6

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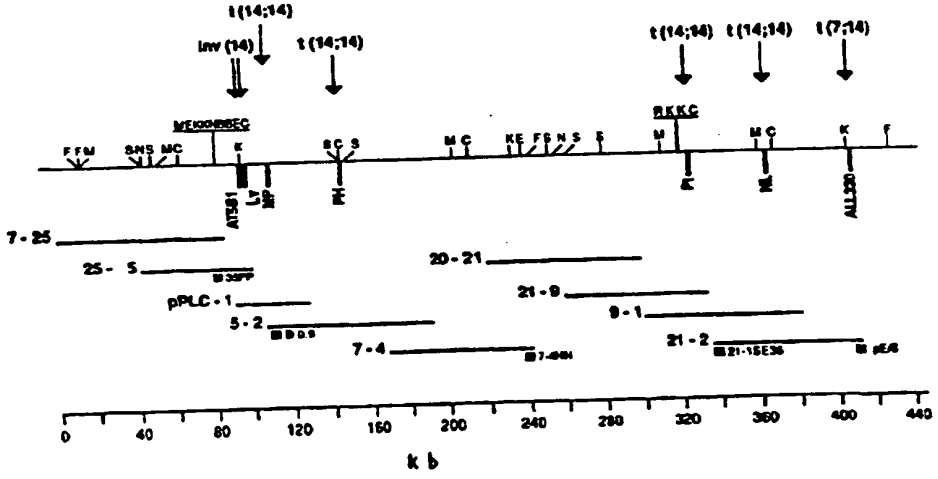

(57) Abstract

Several breakpoints associated with T-cell neoplasia have been mapped to the tcl-1 locus. Accordingly, probes have been made available that can be used for the diagnosis of a chromosomal abnormality occurring in the tcl-1 locus, wherein the or each probe hybridises with human chromosome 14q32.1 in an area of 300-450 kb spanning the AT581 and ALL320 breakpoints and spanned by the proximal probe 7-25 (centromeric) and the distal probe 21-2 (telomeric).

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DIAGNOSTIC PROBES AND THEIR USE IN DETECTING HUMAN CHROMOSOMAL ABNORMALITIES

Field of the Invention

This invention relates to probes for human chromosome 14 subband q32, an area involved in human tumourogenesis, especially of the haematopoietic type.

Background of the Invention

Most human tumours exhibit non-random chromosomal alterations. This is especially seen in hematological neoplasms, where these alterations are observed cytogenically as chromosomal translocations, inversions, deletions and trisomies. It has been shown, during the past decade, that such an alteration is one of the events responsible for the neoplasia. One of the most important genetic mechanisms in the activation of tumour phenotype, is a chromosome translocation. In this type of genetic alteration, two pieces of different chromosomes are joined. If this process involves genes, such as the so-called cellular proto-oncogenes, or genes important for the control of growth, such as cycline, or genes involved in programmed cell death, and destruction or deregulation of their normal function ensues as a consequence of a chromosomal translocation, then a normal cell undergoes a malignant transformation.

In lymphatic neoplasms of B and T origin, a chromosomal translocation is, in the great majority of the cases, the result of a specific mistake that joins sequences of a gene coding for one of the receptor of these cells, i.e. immunoglobulins (Ig) for B cell or T-cell receptors, TCRs for T cell, and a gene on a different location such as c-myc, bcl-2 or all-1.

In T-cell tumours, these chromosomal translocations are usually associated with the faulty joining of genes during the physiological process, leading to the recombination of the TCRs genes located on chromosome 14q11 (TCRα), 7q35 (TCRβ) and 7p15 (TCRγ). On the other side, the loci involved in these rearrangements are located on

chromosomes 8q23 (c-myc), 14q32.1 (tc1-1), 11p15 (tc1-2), 10q23(tc1-3).

The area on chromosome 14q32.1 is highly involved in chromosomal rearrangements of several T-cell neoplasias. Such rearrangements take the form of invl4(q11;q32) inversion or t(14;14)(q11;q32) or t(7;14)(q35;q32) translocation. They are observed in the malignancies of T-Prolymphocytic Leukaemia (T-PLL), in chronic and acute T-cell leukaemias arising in patients with the immunodeficiency syndrome Ataxia-Telangiectasia (AT), and in non-malignant clonal expansion of T cells of patients with AT. Kamada et al (1992) Cancer Res. 52(6):1493 report that such rearrangements occur in 28% of cases of Adult T-cell Leukaemia (ATL) associated with HTLV-I infection.

Several chromosomal translocations in the tc1-1 locus, i.e. the area 14q32.1, have been cloned and characterised. More specifically, the breakpoints identified as Lv and Pt; AT5B1; MP; PH; NL; Pt; and ALL320 are respectively disclosed by Mengle-Gaw et al (1987) EMBO Journal 6:2273-2280; Baer et al (1988) PNAS USA 84:9069-9073; Russo et al (1988) Cell 53:137-144; Davey et al (1988) PNAS USA 85:9287-9291; Russo et al (1989) PNAS 86:602-606; and Bertness et al (1990) Cancer Genet. Cytogenet 44:47-54. Further details are given in the following Table:

Table 1

Case	Probe	Disease	Cytogen
Lv	ND	T-CLL	inv(14)(q11;q32)
AT5B1	AT5B1mer	T Leukaemia in AT	inv(14)(q11;q32)
MP	PB0.9, pMTBE1.4	T Leukaemia in AT	t(14;14)(q11;q32)
PH	IKIMER	T Leukaemia in AT	t(14;14)(q11;q32)
NL	IKIIMER	T-CLL	t(14;14)(q11;q32)
Pt	PTmer	T-PLL	t(14;14)(q11;q32)
ALL320	pE/S, pB15ES	T Leukaemia in AT	t(7;14)(q35;q32)

All of the translocations and inversions cloned have been shown to map by in situ hybridization techniques to chromosome 14q32.1. However, except for two disclosed by Mengle-Gaw et al (1988) PNAS USA 85: 9171-9175, no molecular linkage has been found, nor has a gene has been identified in this locus. This has made probes developed in the area 14q32.1 unsuitable as diagnostic tools for the detection and monitoring of the tumours.

The rearrangement involved in these chromosomal translocations is situated at the subband q32.1 of human chromosome 14. Also the gene for the heavy chain of Ig (IgH) is situated on chromosome 14, but as subband 14q32.3. The physical distance between the two loci is calculated to be at least 10,000 kb.

Inv(14) inversion has been also described in the cell line SupT1 and, sporadically, in 15% of normal B and T lymphocytes. In the case of SupT1, the molecular rearrangement involves the area 14q11 (TCRα) and 14q32.3 (IgH), resulting from a mistake during the physiological process of recombination that occurs in these loci. The translocation observed in this case is a manifestation of interlocus recombination between T-cell receptor and immunoglobulin heavy chain gene complexes.

There is now ample consensus on the hypothesis that these chromosomal translocations are different in terms of tumorigenicity from the translocations occurring in the area 14q32.1. The latter have the potential to be oncogenic; those occurring at 14q32.3, between T-cell receptor and immunoglobulin heavy chain gene complex, are not oncogenic.

Cytogenetically, it is not easy to distinguish between the two alterations. Also, since the positions of the breakpoints in the areas 14q32.1 and 14q32.3 are not clustered, it is very difficult to apply conventional techniques, such as Southern blotting, to detect rearrangements.

Summary of the Invention

As described in more detail below, the area of 14q32.1 has been mapped. It has been found that a region of 300 kb is the target of the chromosomal rearrangements occurring in this area. By using a P1 cloning system (NEN-DUPONT) that is commercially-available, several clones have been isolated (see Fig. 1). They and analogous clones can be used:

a) to diagnose chromosomal translocations occurring in the tcl-1 locus at band 14q32.1. The usage of these clones can show directly a point of breakage, by splitting the hybridisation between the two parts of the different chromosomes involved in the rearrangements. This can be done, either by using some or all of the probes together or by using the more proximal/centromeric (7-25) and the more distal/telomeric (21-2); in this latter case, if these clones are stained with two different colours, the split may be made more easily evident.

b) to differentiate between the chromosomal inversions inv(14)(q11;q32.1) occurring in the area 14q32.1 and those occurring in an area telomeric to this, as for example the common inversion sporadically observed in normal lymphocytes. In this case, if the inversion involves the 14q32.1 area, a more proximal clone (7-25) or more distal clone (21-2) will be moved near the centromere of the chromosome 14 near the area 14q11.

c) to diagnose those cases in which a chromosomal translocation, as in the case of neoplasia carrying a t(7;14)(q11;q32) chromosomal translocation (see Russo et al, Cell, supra) or carrying a t(14;14)(q11;32) chromosome translocations (see Russo et al, PNAS, supra) is associated to an inversion with duplication of the tip of 14q32. In this case, by using one of the clones, a double hybridisation can be seen near the 14q32.1 area, since this is duplicate.

d) in Southern blotting techniques (conventional and Pulse Field) against DNA from samples using a normal DNA as competitor.

Brief Description of the Drawings

In the accompanying drawings:

Fig. 1 shows the genomic organisation of the tcl-1 locus on chromosome 14q32.1. Restriction sites are given for B=BssHII, C=ClaI, E=EagI, F=SfiI, K=KspI, M=MluI, N=NotL, R=NruI, S=SalI. Ranges covered by isolated P1 clones or cosmid clone pLC1 are shown by horizontal bars with their names. Arrows and solid lines illustrate the position of chromosome breakpoints described in Table 1 and in the text. Shadowed boxes represent probes used and described in the text, for isolation of P1 clones and for hybridisation.

Fig. 2 is a schematic representation of the chromosomal rearrangements involving the tcl-1 locus on chromosome 14q32.1 and the TCR α-chain locus (TCRA) chromosome 14q11.2. The shadowed arrows represent the orientation of the TCRα while the shadowed arrows represent an arbitrary orientation of tcl-1. Part a shows normal chromosome 14. b is a representation of an inv(14) chromosomal inversion. c is a representation of a t(14;14)(q11;q32) classical chromosomal translocation. d is a representation of a t(14;14)(q11;q32) chromosomal translocation with an inversion and duplication of 14q32.1->14qter. Vα, Jα and Cα represent variable joining and constant gene segments of TCRA.

Description of the Invention

The term "clone" as used herein refers to a specific human DNA fragment located on human chromosome 14q32.1, e.g. at least 10 or 20 kb, preferably between 40 and 100 kb long. Such a clone may be inserted in the vector pNS582tet14Ad10 of the P1 cloning system. The numbers 7-25, 25-5, 4-25, 5-2, 20-21, 21-9, 9-1 and 21-2 serve as identification of the clones that have been made and are shown in Fig. 1; these clones cover an area of

approximately 420 Kb on human chromosome 14q32.1. These clones are grown on E. Coli NS3145 in L-Broth containing 25 μg of kanamycin and can be prepared by conventional DNA techniques such as alkaline lysis followed by centrifugation on a caesium chloride gradient, as described in the Cold Spring Harbor Lab. Manual.

The clones of the invention may find utility as diagnostic probes to be used in techniques of in situ hybridisation. Such techniques include those described in WO-A-92119775 and US Patent Application Serial No. 08/145,908, filed Oct. 29, 1993, the contents of which are incorporated by reference herein; and these in which a DNA fragment is labelled, either by radioactive compounds, as described by Russo et al, Cell, supra, or by non-radioactive compounds such as fluorescent compounds (see Cherif et al (1990) PNAS USA 87:6639-6643), followed by hybridisation to metaphase chromosomes. Especially when labelling of the probes with non-radioactive compounds, as for example in fluorescent in situ hybridisation (FISH), these clones can be particularly useful, since it is well known that this technique works better when using probes containing large fragments, more than 10 kb long.

In particular, the present invention relates to isolation, identification and production of a unique set of clones which can potentially be used in the diagnosis, prognosis and monitoring of certain TCR-related aberrations which occur in certain leukaemias and related diseases.

Isolation of these clones follows chromosome walking of the 14q32.1 area, with the aim of defining the organisation and physical linkage of the reported breakpoints. The molecular cloning of several hundred kilobases of germline sequences in the 14q32.1 area has allowed further characterisation of the locus tcl-1 involved in various T-cell leukaemias. Starting from two previously cloned breakpoints, and using a chromosome walking technique with a P1 cloning system, a germline area of 450 kb on chromosome 14q32.1 has been defined that

encompasses the two breaks adjacent to t(14;14)(q11;q32) and t(7;14)(q35;q32) chromosome translocations occurring in two T-cells leukaemias of patients with AT. Several other cloned breakpoints have also been found in this area, indicating that a cluster region of c. 300 kb is the target of the chromosomal rearrangements involved in haematopoietic malignancies such as T-PLL, T-CLL and T-ALL of patients with and without AT.

These arrangements can take the form of inv(14)inversion, or t(14;14)(q11q32) or t(7;14)(q35;q32) translocation, and all of them cytogenetically map to band 14q32.1. Two of such rearrangements, namely the two chromosome inversions Lv and AT5B1, were already described Mengle-Gaw, supra, to be 2 kb apart. It is also evident from the results now found that, on a molecular level, the translocations and inversions occur in the same area, since the two above-mentioned inversions are located at only 10 kb from another malignant T-cell AT clone carrying a t(14;14) chromosomal translocation. This finding strongly suggests that both inversion and translocations occur by the same mechanism and have the same target on 14q32.1.

All the rearrangements discussed herein involve chromosome segments at 14q11 and 14q32.1. In the 14q11 area, they affect the TCRα chain, that spans an area of almost 80 kb, and usually involve Jα segments or pseudo-segments and whose orientation on 14q11 is centromere->Vα->Jα->Cα->telomere. In the 14q32 area, they involve the area shown in Fig. 1, with an orientation: centromere->AT5B1->B0.9->pE/S->telomere.

Without wishing to be bound by theory, the following model of activation is possible: an inversion (see Fig. 2b), as in AT5B1 and Lv, will bring the Jα-Cα region 5' to the tcl-1 locus, with an orientation of the newly formed locus at 14q32.1 that will be centromere->Cα->Jα->tcl1->telomere. A translocation (as observed in the case of Pt, for example) will instead bring the incoming 14q11

sequences, 3' to the tcl locus, in the orientation centromere->tcl1->Jα->Cα->telomere (see Fig. 2c).

However, the situation is complicated by the observation that some of the translocations in this area are accompanied by another abnormality, such as an inversion with duplication, involving the tip of chromosome 14q32, as for example in the cases of the AT malignant T cell clones MP and ALL320. The duplication event, whose significance, extension and temporal occurrence remains yet unrevealed, does not affect the position of the breakage on chromosome 14q32.1. This is demonstrated by the proximity of the two inversions (AT5B1 and Lv) to the translocation 513. Structurally, an inversion with duplication (see Fig. 2d) will behave in terms of the juxtaposition of TCR elements as just observed for the inversion cases, suggesting also in this case the same mechanism of activation.

The experimental procedures that have been adopted will now be described, by way of illustration ("standard procedures" are these in Molecular Cloning: A Laboratory Manual, unless otherwise stated).

Construction, screening of a human P1 library

The P1 library was constructed using a commercial kit (Dupont-NEN). Briefly, human placental DNA was partially digested to an average size of 80-100 kb and ligated into a BamHI-restricted site of the tetracycline (tet) resistance gene in the vector pNS582tet14Ad10, according to the procedure described by Stenberg (1990) PNAS USA 87:103-107. The DNA was then packaged using commercial packaging extracts and plated on the bacterial strain NS3145 on L-kanamycin (50 μg/ml) agar at a density of approximately 5 x 10^3/plate. 7-10 x 10^4 colonies were obtained and amplified by each single plate according to standard protocols. A cosmid library was constructed and screened according to the procedure described by Isobe et al (1988) PNAS USA 85: 3933-3937. Screening was performed either by

standard colony hydridisation with the probes of interest or by PCR amplification.

Standard DNA methods

Restriction and modifying enzymes were purchased from different manufacturers and were used as specified from the vendor. High molecular weight DNA and RNA were prepared from several human cell lines such as Jurkat 2, Molt 4, K562, Daudi, ALL 380, CEM normal human thymus, from the somatic cell hybrids containing the der 14q+ CMC513AC3B10 of the case MP and 602BC1BE1 of the case ALL 320 as described by Russo et al, supra, from normal tissues or cell lines of different specifies.

Plasmid DNAs were isolated by the alkaline lysis method, followed by separation on a caesium chloride gradient.

Restriction-digested DNAs were fractionated by standard agarose gel electrophoresis or by Exagonal-Field-Electrophoresis using apparatus available from LKB-Pharmacia. In the latter case, samples were first electrophoresed at 160 Volts with a pulse time of 1 second for 9 has followed by a pulse time of 2.5 seconds for 9 has. DNAs and RNAs were then blotted and hybridised following standard procedures.

Oligonucleotides and probes

Oligonucleotide pairs used for PCR on the P1 library are:

MT5'&MT3' CTGCCTCATTGGCCTTCCAA/GACAAGAAGTGCTGGGATTC from sequence adjacent to the breakpoint MP;

21-2HH1 TTGACATGATATAATGGCGG/CAGTTCCTAGGTACGTGAC derived from the left end sequence of clone 21-2;

5-2/5'&5-2/3' TGTAGGGATTGTGCTTCAGC/TACTATGGGTGCTGCCCATC derived from the right end sequence of clone 5-2;

9-1KK/5'&9-1KK/3' CTACACCAGGGAGGGGCA/TGCCACCTCCTACACCAT derived from internal sequences of clone 9-1;

pLC1/5'&pLC1/3' AACTCTGAAGTTCTCCTT/GGGCAGAGGCTGAGACTT derived from the left end sequences of cosmid clone pLC1;

25-5/5'&25-5/3' GCAGGACAAGGCGGACTA/AGATTCACTCCCGCCAAG derived from the left end sequences of the clone 25-5.

Oligonucleotides used from other previously cloned sequences of T-cell malignancies involving the 14q32.1 area (see also Table 1) are ATTTACAAATTGCATCTGAG (IKI), TGCAGAAAGGAAACTGGGTA (PT5), TCTCTTACACCAAACAGTCATCA (AT5B1) and AGGTCACCTGAACTAAGGAGGGA (IKII).

The pBR322 fragments SalI-NruI (325 bp) and BamHI-EcoRV (188bp), flanking the bacteriophage P1 cloning site BamHI, were used to detect left or right ends of newly isolated P1 human clones.

Probes B0.9 and pE/S have been described by Russo et al, supra, and are adjacent respectively to the t(14;14) translocation of MP and to the t(7;14) translocation of ALL320 on chromosome 14q32.1.

PCR

Amplification by PCR was carried out for 25 cycles under the following conditions: denaturation at 94°C for 1 min., annealing at 50-61°C (depending on Tm of the primers used) for 1 min., and elongation at 72°C for 2 min was carried out on a Hybaid thermocycler.

DNA sequencing and analysis

DNA sequencing performed on restriction fragments cloned into pUC18/19 or pBSII plasmids by the dideoxynucleotide chain-terminator method using the Sequenase sequencing kit (USB). Sequences were analysed with Wisconsin Genetics Group software and with the commercial software Dnasis (Hitachi Co. Ltd.).

P1 library screening by PCR

A human genomic library was prepared in bacteriophage P1 cloning vector, which allows for an average insert size of 80 kb, as described above. A PCR-based approach was used to screen the library, with the intent to minimise the manipulations and the long-term storage problems of recombinant libraries. Briefly, the primary library was plated out at a density of approximately 5 x 10^3/plate, and each plate was separately amplified and stored in 20%

glycerol at -80°C. An aliquot of each stock was later inoculated and grown in 5 ml of LB supplemented with kanamycin. DNA was extracted and it served as a template for PCR-based screening. At each screening, the 20 different DNAs were exposed to 25 cycles of PCR with the appropriate primers. Following identification of positives, only the sub-libraries of interest were plated and screened with the required probe. This approach made is possible to overcome some of the shortcomings engendered by the low copy number of the P1 system and by repeat screening of amplified libraries.

Cloning of the genomic tcl-1 locus

In order to obtain a complete physical map of the 14q32.1 area, a chromosome walking was started from two sites (see Fig. 1) previously characterised by Russo et al, supra: the breakpoint (ALL320) of a t(7;14)(q35;q32) chromosome translocation of a T-ALL patient with AT, and the breakpoint (MP) of a t(14;14)(q11;q32) chromosome translocation of another T cell leukaemia patient with AT. Primers and probes (see Table 1 and Fig. 1) derived from sequences adjacent to the two breakpoints were used to screen the human P1 genomic library. Two positive clones were isolated in the first round of walking, the 21-2 of 75 kb and the 5-2 of 83 kb containing germline sequences on 14q32.1. The results of the restriction enzyme mapping of the two clones are shown in Fig. 1.

A probe 21-2SE53 corresponding to the left end of 21-2 and the pE/S probe corresponding to the right end of 21-2 failed to hybridised to clone 5-2 (Fig. 1), indicating that the two P1 clones were not overlapping. In situ hybridisation, performed with the P1 clone 21-2 on the somatic cell hybrid 513AC3B10 containing a 14q+ from the case MP, showed three separate hybridisations, two close to the translocation breakpoint and one at a very telomeric site. This result is consistent with the presence of an inversion with duplication of the telomere of chromosome 14q+, as already observed in this and other similar cases,

suggesting that the orientation of the two probes on the chromosome is the following: centromere->B0.9->pE/S-> telomere.

In order to join the gap between the two clones, chromosome walking was performed. To generate new probes for the next round of screening, the sequences at the ends of the two human inserts were identified by hybridisation with probes flanking the cloning site. For this purpose there were used on one side the pBR322 SalI-NruI fragment and on the opposite side the BamHI-EcoRV fragment. The end fragments, containing vector and human sequences, were then subcloned, mapped and sequenced to generate new germline 14q32.1 primers and probes.

The cloning proceeded until two P1 overlapping clones coming from opposite directions (7-4 and 20-21) were found. These results were confirmed by hybridisation of the probe 7-4HH (see Fig. 1) to restricted DNA from clone 20-21, indicating that the gap between the two breakpoints had been covered.

Only on one occasion was no overlapping clone found, using a probe from clone 5-2, and to further proceed into the walking a cosmid library was screened. The clone pLC1 was hence isolated from this library (Fig. 1).

A total of 12 clones were isolated, some of which were almost totally overlapping (and are therefore not shown), to cover an area of 420 kb (Fig. 1). The two breakpoints used as starting sites are 300 kb apart, confirming the involvement of a very large area in the chromosome 14q32.1 translocations. Whereas Russo et al, PNAS, supra reported that the two probes pE/S and B0.9 could be contained within a 250 kbp SfiI restriction fragment, cloning data indicate that these two probes are on different SfiI fragments approximately of 240 kb (B0.9) and 215 kb (pE/S).

To ascertain that no interchromosomal rearrangements had taken place during the cloning process, the primers employed for chromosome walking were also tested for amplification on DNA derived from a rodent x human somatic

cell hybrid 5263C17, which contains a single human chromosome 14q+(14pter->14q32.1::Xq13->xqter); see Russo et al, Cell, supra. Every primer pair tested was found to amplify sequence from this DNA, indicating that the clones had not undergone rearrangement during the cloning and the amplification procedures.

Mapping of four other breakpoints

To understand if the cloned area was the target of other breakpoints observed in similar T-cell neoplasia, sequences from other 14q32.1 cloned arrangements, whose molecular linkage was not previously shown, were hybridised to the described germline clones. Oligonucleotides derived from sequences adjacent to the breakpoints of three t(14;14)translocations, namely Pt, PH and NL (see Table 1), were synthesised and hybridised to the P1 clones. Pt mapped on clone 9-1 about 80 kb centromeric to pE/S, whereas PH mapped on clone 5-2 about 25 kb telomeric to B0.9, and NL on clone 21-2 about 45 kb centromeric to pE/S. Hybridisations were obtained by Southern blotting and results are shown in Fig. 1.

Furthermore, it was of interest to ascertain if the breakpoints of reported cases of inv(14)(q11;q14) did also physically map to the same area. Inv(14) chromosomal rearrangements have been observed in several patients with T-CLL, T-PLL and T-cell lymphoma, and their breakpoints cytogenetically mapped on band 14q32.1. For this purpose the oligo AT5B1, originating from the inv(14) AT5B1, was hybridised to a Southern blot of the P1 clones and of the cosmid clone pLC1. While no hybridisation was observed with DNA from the P1 clones, the oligo hybridised to the cosmid clone pLC1. Fine restriction enzyme mapping allowed location of this sequence 7 kb centromeric to B0.9. This inversion had also been reported to be only 2.1 kb telomeric Lv; see Mengle-Gow, supra. All these results taken together indicate that the chromosomal rearrangements t(14;14) and inv(14) are physically linked and the area cloned is comprehensive of the entire tcl-1 locus.

CLAIMS

1. Use of one or more DNA probes for the diagnosis of a chromosomal abnormality occurring in the tcl-1 locus, wherein the or each probe hybridises with human chromosome 14q32.1 in an area of 300-450 kb spanning the AT581 and ALL320 breakpoints and spanned by the proximal probe 7-25 (centromeric) and the distal probe 21-2 (telomeric).

2. Use of two probes according to claim 1, the probes being respectively distal and proximal.

3. Use of two overlapping probes according to claim 1.

4. Use of a probe according to claim 1, for the diagnosis, by double hybridisation, of a chromosomal translocation associated to inversion with duplication.

5. Use according to any preceding claim, wherein the or at least one probe spans the AT5B1, Lv, MP, PH, Pt, NL or ALL320 breakpoint.

6. Use according to any preceding claim, wherein the or each probe is more than 10 kb long.

7. Use according to claim 6, wherein the or each probe is between 40 and 100 kb long.

8. Use according to any preceding claim, wherein said area is as shown in Fig. 1.

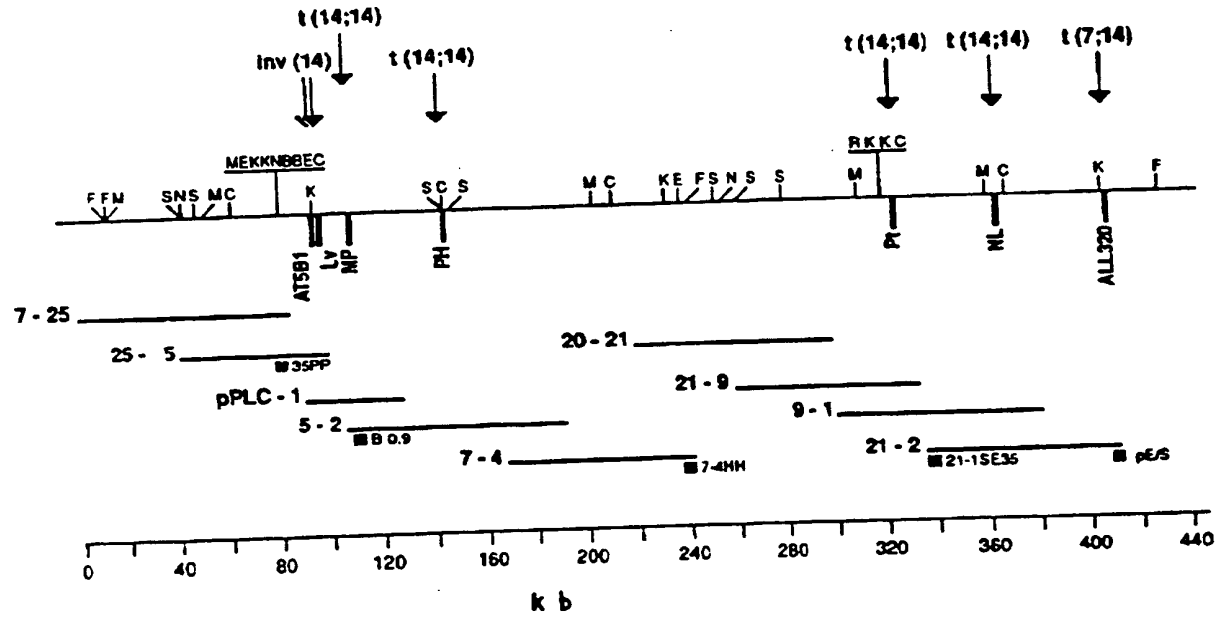

Fig. 1

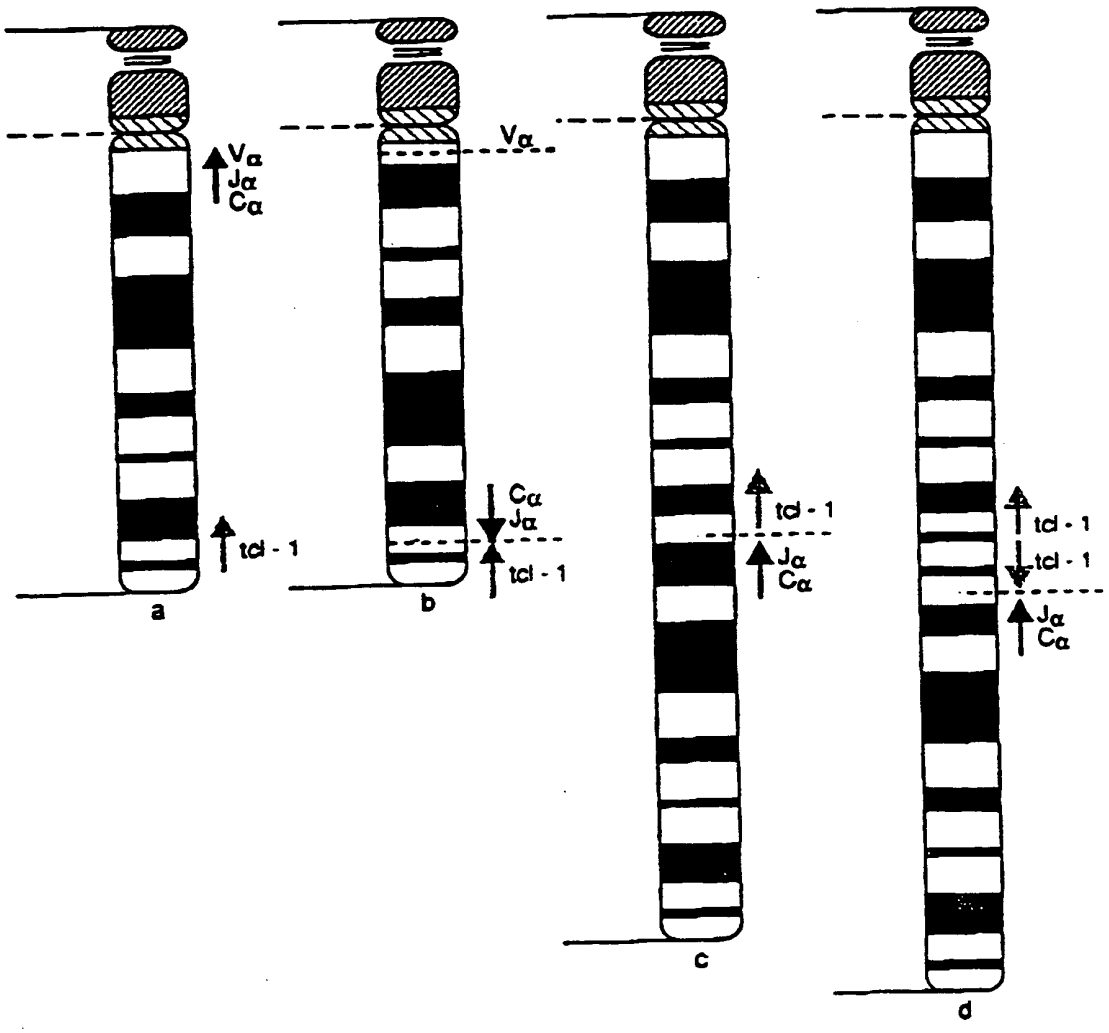

Fig. 2

INTERNATIONAL SEARCH REPORT

Internat ·l Application No
PCT/EP 94/01183

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROC. NATL. ACAD SCI. vol. 86 , January 1989 , NATL. ACAD SCI., WASHINGTON, DC, US; pages 602 - 606 G. RUSSO ET AL. 'Molecular analysis of a t(14;14) translocation in leukemic T-cells of an ataxia telangiectasia patient' cited in the application see page 605, left column, line 2 - line 12; figures 2,4,6 see page 605, right column, line 36 - page 606, left column, line 3 --- -/--	1,2,5,8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 1994	26 -08- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hornig, H

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INTERNATIONAL SEARCH REPORT

Interna' ul Applicanon No
PCT/EP 94/01183

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBO JOURNAL vol. 6, no. 8 , August 1987 , IRL PRESS LIM., OXFORD, ENGL.; pages 2273 - 2280 L. MENGLE-GAW ET AL. 'Human T-cell tumours containing chromosome 14 inversion or translocation with breakpoints proximal to immunglobulin joining regions at 14q32' cited in the application see page 2273, paragraph 2 - page 2278, paragraph 1 ---	1,5,8
X	PROC. NATL. ACAD SCI. vol. 84 , December 1987 , NATL. ACAD SCI., WASHINGTON, DC, US; pages 9069 - 9073 R. BAER ET AL. 'The breakpoint of an inversin of chromosome 14 in a T-cell leukemia: Sequences downstream of the immunglobulin heavy chain locus are implicated in tumorigenesis' cited in the application see page 9070, left column, line 33 - page 9072, left column, line 16 ---	1,5,8
X	CELL vol. 53 , 8 April 1988 , CELL PRESS, CAMBRIDGE, MA,US; pages 137 - 144 G. RUSSO ET AL. 'Molecular analysis of a t(7;14)(q35;q32) chromosome translocation in a T cell leukemia of a patient with Ataxia Telangiectasia' cited in the application see page 138, left column, paragraph 2 - page 141, right column, paragraph 1 ---	1,5,8
X	PROC. NATL. ACAD SCI. vol. 85 , December 1988 , NATL. ACAD SCI., WASHINGTON, DC, US; pages 9171 - 9175 L. MENGELE-GAW ET AL. 'Analysis of a T-cell tumor specific breakpoint cluster at human chromosome 14q32' cited in the application see page 9171, right column, paragraph 3 - page 9175, left column, paragraph 2; figures 1,4 --- -/--	1,5,8

INTERNATIONAL SEARCH REPORT

Internat ·l Applicanon No
PCT/EP 94/01183

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CANCER GENET. CYTOGENET. vol. 44, no. 1 , January 1990 , ELSEVIER SCIENCE PUBL. CO., INC.,NEW YORK, US; pages 47 - 54 V.L. BERTNESS ET AL. 'Characterization of the breakpoint of a t(14;14)(q11.2;q32) from the leukemic cells of a patient with T-cell acute lymphoblastic leukemia' cited in the application see page 48, paragraph 6 - page 51, paragraph 1; figure 3	1,5,8
A	WO,A,91 00364 (TEMPLE UNIVERSITY) 10 January 1991 the whole document	1-8
A	CANCER REASERCH vol. 52, no. 6 , 15 March 1992 , WARVERLY PRESS INC., BALTIMORE,US; pages 1481 - 1493 N. KAMADA ET AL. 'Chromosome abnormalities in adult T-cell leukemia/lymphoma: A karyotype review committee report' cited in the application the whole document	1-8
P,X	PROC. NATL. ACAD SCI. vol. 90, no. 20 , 15 October 1993 , NATL. ACAD SCI., WASHINGTON, DC, US; pages 9275 - 9279 L. VIRGILIO ET AL. 'Chromosome walking on the TCL1 locus involved in T-cell neoplasia' see page 9276, right column, line 25 - page 9278, left column, line 4; figure 1	1,5,8

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat[illegible] Application No
PCT/EP 94/01183

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9100364	10-01-91	AU-A-	5932790	17-01-91
		CA-A-	2063409	04-01-91
		EP-A-	0480970	22-04-92
		JP-T-	5500302	28-01-93
		US-A-	5242795	07-09-93